

## EFFICACY OF FEED ADDITIVES ON MEAT QUALITY AND NUMBERS OF FAECAL BACTERIA IN GROWING RABBITS

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**ABSTRACT:** The aim this study was to evaluate the effect of 6-week application of zinc protein hydrolysate Bioplex-Zn to feed mixtures for rabbits and combinative application with 0.1% concentration thyme plant extract applied into water during the growing period on the selected parameters of meat quality and the caecal and faecal microbiota. Experiment was realized on 96 post-weaned rabbits (meat line M91 a P91). They were randomly divided into 4 similar experimental groups (EG), with 24 animals in each group. The rabbits in the group 1EG and 3EG were fed the same commercially available diet with no zinc additive. The feed mixture in the 2EG and 4EG was additionally administered with a dose of 33.3 g Bioplex-Zn per 100 kg (this is addition of 50 mg Zn/kg feed mixture). The rabbits in the group 3EG and 4EG received a dose with (0.1% *Thymus vulgaris* L. plant extract) applied into drinking water. Their beneficial effect was tested in a fattening experiment during 42 days. At the end of the experiment, 6 animals from each group were slaughtered. Meat quality was analysed from MLD (*Musculus longissimus dorsi*) samples. The amino acids and fatty acids contents were examined, showing statistically significant changes ( $P \leq 0.05$ ). The caecal chymus were collected to detect the total bacterial content. The faecal samples were collected on day 1 and 42. To check microbiota, sampling of faecal mixtures per each group was performed at the start and end of the experiment. Samples were treated using the standard microbiological dilution method by plating appropriate dilutions on the selective media according to ISO. Microbiota was expressed in CFU.g<sup>-1</sup>. The treatment did not have a negative influence on the pH, colour, water-holding capacity, protein and fat contents or energetic value of the rabbit carcass. The beneficial effect of combination plant extract *Thymus vulgaris* and zinc additive administration was manifested by the anti-coccidian effect in rabbits.

**Key words:** rabbit, microbiota, bioplex-zinc, meat quality, amino acids

### INTRODUCTION

Zinc is an essential nutrient in human and animal nutrition, that is required for numerous metabolic functions, and zinc deficiency results in growth retardation, cell-mediated immune dysfunction, and cognitive impairment. The quantity of a certain mineral in food is not as important as its availability for the animal. This availability

varies to a high degree, depending on numerous factors such as: their form in the food, the phase of development of plants, the presence of other minerals and components in the foods which bind them and make them unavailable, as well as the age and sex of the animal. The need for Zn by most animals is based on its influence on enzymes and proteins and their activities, that are linked to vitamin A synthesis, carbon dioxide (CO<sub>2</sub>) transport, collagen fiber degradation, free radical destruction, membrane stability of red blood cells, metabolism of essential fatty acids, carbohydrate metabolism, protein synthesis, metabolism of nucleic acids, among others (Powell, 2000; McCall et al., 2000; Stefanidou et al., 2006; Rubio et al., 2007; Suttle, 2010; Chrastinová et al., 2016). Zinc is a trace element important in nutrition; thyme possesses antibacterial activity. New and safe antimicrobial agents are searched for to prevent and/or overcome infections. Prevention and treatment of clostridial infections by natural substances or phyto-additives are important, because of animal mortality and economic losses.

The aim of this study was to examine the effects of combinative orally administered zinc from organic source Bioplex-Zn and 0.1% dose of *Thymus vulgaris* L. plant extract applied into drinking water on growth performance and their influence on the parameters of rabbit meat quality and monitor the microflora in faecal and caecal samples.

## MATERIAL AND METHODS

A total of 96 post-weaned rabbits (aged 5 weeks, both sexes, meat line P91 and M91) were divided into 4 groups with 24 animals in each group, 6 replicates. The rabbits were kept in standard cages, two animals per cage. The cages allowed faeces separation. A cycle of 16 h of light and 8 h of dark was used throughout the experiment. The heating and forced ventilation systems allowed the building air temperature to be maintained within  $24 \pm 4^{\circ}\text{C}$  through the experiment. The relative humidity was about  $70 \pm 5\%$ . All care and experimental procedures were approved by the Slovak Veterinary and Food Administration. To the feed mixture in the 2EG and 4EG, a dose of 33.3 g Bioplex-Zn (is a product of Alltech, USA) was additionally administered per each 100 kg (this is addition of 50 mg Zn/kg into feed mixture). The rabbits in the group 3EG and 4EG received a dose with *Thymus vulgaris* L. (0.1% plant extract; a product of Calendula s.r.o. Slovakia) applied into drinking water. The animals were fed complete pelleted feed (pellets of 3.5 mm in diameter) *ad libitum* and had free access to water. The ingredients and chemical composition of this diet is presented in Table 1.

Chemical analyses were conducted according to AOAC (2005). Body weight and feed consumption were measured every week during the experiment. Mortality and morbidity were also recorded in the groups daily, over the entire period of the experiment. The fattening experiment lasted for 42 days. Sampling of faeces was performed on day 1 (start of the experiment, faecal mixture of 96 animals in 10 samples) and at the end of the experiment (6 weeks of additive application, 5 faecal mixtures per each group).

The samples for microbiological analyses were treated by the standard microbiological method according to ISO (International Organization for Standardization) using appropriate dilutions in Ringer solution (pH 7.0, Oxoid Ltd., Basingstoke, Hampshire, England) with appropriate media.

Table 1. Ingredients and chemical analysis of the granulated diets for growing rabbits

Ingredients/Nutrients	%	g.kg <sup>-1</sup> dry matter	Basal diet (BD)	BD diet supplemented with Bioplex Zinc
Lucerne meal	36	Crude protein	172.54	172.55
Extracted sunflower meal	5.5	Crude fibre	164.73	169.07
Extracted rapeseed meal	5.5	Fat	41.10	41.17
Wheat bran	9	Ash	82.95	82.59
Oats	13	Starch	183.29	182.19
Malt sprouts	15	Organic matter	917.05	917.41
DDGS	5	ADF	209.46	214.13
Sodium chloride	0.3	NDF	334.36	346.04
Mineral - vitamin mixture*	1.7	Calcium	6.52	6.64
Barley grains	8	Phosphorus	6.34	6.53
Bioplex-Zn	1	Zinc (mg.kg <sup>-1</sup> )	106.56	161.53
Limestone		ME (MJ.kg <sup>-1</sup> )	10.99	10.84

\*Premix contains per kg: calcium, 6.73 g; phosphorous, 4.13 g; magnesium, 1.90 g; sodium, 1.36 g; potassium, 11.21 g; iron, 0.36 g; zinc, 0.13 g; copper, 0.03 g; selenium, 0.2 mg. Vitamin mixture provided per kg of diet: Vitamin A 1500000 IU; Vitamin D3 125000 IU; Vitamin E, 5000 mg; Vitamin B1, 100 mg; Vitamin B2, 500 mg; Vitamin B6, 200 mg; Vitamin B12, 0.01 mg; Vitamin K3, 0.5 mg; biotin, 10 mg; folic acid, 25 mg; nicotinic acid, 4000 mg; choline chloride, 100000 mg. DDGS: dried distillers grains with solubles; ADF: Acidodetergent fibre; NDF: Neutraldetergent fibre

The experiment was performed in cooperation between the Institute of Animal Physiology, Slovak Academy of Sciences, Košice and the National Agricultural and Food Centre - Research Institute for Animal Production Nitra. The zootechnical parameters were monitored daily. Rabbits were slaughtered on day 42 of experiment (6 animals from each group for carcass evaluation) before morning feeding for determination of physico-chemical characteristics in (*Musculus longissimus dorsi*) MLD muscles; they were stunned by electronarcosis (90 V for 5 s), immediately hung by the hind legs in the processing line and quickly bled out by cutting the jugular veins and the carotid arteries. The fur and viscera were removed. The dressing percent was calculated as the ratio of dressed weight to the live weight. Organs like kidney, lung, heart and liver were removed and weighed individually. Carcass data were obtained using the method described by Rafay et al. (1999), taking into account the harmonizing criteria according to Pla and Dalle Zotte (2000). The MLD collected (50g) immediately after death and stored 24 h at 4°C and the physical chemical characteristics and chemical composition were determined by standard methods (STN 570185). The content of water, protein and fat were estimated using a FoodScane™ - Meat Analyser (FOSS, Denmark) by method FT IR (Fourier Transform infrared Spectroscopy); expressed in g.100g<sup>-1</sup>, from these values, the energy value was calculated according to the equation:

$$EV (\text{kJ}.100\text{g}^{-1}) = 16.75 \times \text{protein content} + 37.65 \times \text{fat content}.$$

The water holding capacity was determined by the compression method at constant pressure (Hašek and Palanská, 1976). The analysed samples (0.3 g in weight) were placed on filter papers (Schleicher and Shuell No. 2040B, Dassel, Germany) with tweezers previously weighed. Together with the papers, samples were sandwiched between Plexiglas plates and then subjected to a pressure of 5 kg for 5 min. The results were calculated from the difference in weight between the slips with aspirating spot and the pure filter paper. The pH value was determined after 24 h (post mortem) using a Radelkis OP-109 (Jenway, England) with a combined electrode penetrating 3 mm into samples. The electrical conductivity ( $\mu\text{S}.\text{cm}^{-1}$ ) is defined as the locations of muscles and was evaluated using PMV 51 (Tecpro GmbH, Germany), colour characteristic were expressed by CIE  $L^*a^*b$  system (lightness- $L^*$ , 0: black and 100: white), (redness and

greenness-*a*\*; yellowness and blueness-*b*\*) using a Lab. Miniscan XE, Lightness measurements at room temperature were also measured. The amino acid composition after acid hydrolysis by 6M HCl and sulphuric amino acids after oxidation hydrolysis was analyzed by ion-exchange chromatography on the AAA iCE 3000 (Ingos Prague, Czech Republic). Caecum and appendix were separated. The caecal chymus were collected to detect the total bacterial content. To check microbiota, sampling of faecal mixtures per each group was provided on the start and end of the experiment. Samples were treated using the standard microbiological dilution method by plating appropriate dilutions on the selective media according to ISO. The bacterial counts were expressed in log<sub>10</sub> colony forming units per g (log<sub>10</sub> CFU.g<sup>-1</sup> ±SD). The results shown are expressed as the average of three parallel replications of each sample.

### **Media and microbial analyses**

To test for microorganisms, the samples (1g) were treated using the standard microbiological dilution method (in Ringer solution, pH 7.0; Oxoid Ltd., Basingstoke, Hampshire, England); 1: 9 dilution, and (100 µl) by plating appropriate dilutions on the selective media according to ISO (International Organization for Standardization). To enumerate enterococci, M-Enterococcus agar was used (NF-V04503, Difco Laboratories, Detroit, USA). Other lactic acid bacteria (LAB) were isolated on MRS agar (ISO 15214, Merck, Germany). Baird-Parker agar supplemented with egg yolk tellurite solution (ISO 21527-1, Difco) was used to enumerate coagulase-positive staphylococci (CoPS). Coagulase-negative staphylococci (CoNS, ISO 6888) were enumerated on Mannitol salt agar (Becton and Dickinson, Cockeysville, USA). Clostridium difficile agar with the supplement SR0096E and 7% (v/v) defibrinated horse blood (SR0050, ISO 15883, Oxoid Ltd., Basingstoke, Hampshire, England) was used to enumerate clostridia. To count coliform bacteria, Mac Conkey agar (ISO 7402, Oxoid) was used. Pseudomonads were isolated on Pseudomonas agar (Biomark). The plates were incubated at 30°C and/or 37°C for 24 - 48 h depending on the bacterial genera. One gram of caecal content and appendix was treated according to the standard microbiological dilution method and plated on the media mentioned above. Randomly picked up representants of selected bacterial groups were confirmed by MALDI-TOF identification system (Bruker Daltonics).

*Eimeria* sp. oocysts were enumerated in the faecal samples microscopically at the start of the experiment (day 0-1) and on the 42nd day of the experiment. The samples were stored at 4°C and then evaluated by the quantitative flotation technique - modified McMaster method (Ministry of Agriculture, Fisheries and Food, Manual of veterinary parasitological laboratory techniques, 1986). The intensity of the infections was estimated from counts of oocysts per 1 g of faeces (OPG).

### **Statistical analysis**

Statistical evaluation of the results was performed by one-way analysis of variance with Tukeys post hoc test with the level of significance set at P<0.05. The results are quoted as means ±SD.

## RESULTS AND DISCUSSION

The trial was carried out from July to September 2017 in the experimental house of the NPPC - Research Institute for Animal Production Nitra, Slovak Republic. Each experimental procedure involving animals was approved by the State Veterinary and Food Institute of the Slovak Republic and Ethic Committee. Experimental animals did not show any health problems during the whole study period. The ability to discriminate among diets varying in Zn concentration has been described for several animal species and nutrients. Zn is important for the organism and has influence on the feed intake; however, there is a lack of data whether rabbits can discriminate among diets differing in mineral content to avoid Zn-deficiency. To the feed mixture in the 2EG and 4EG, a dose of 33.3 g Bioplex-Zn (is a product of Alltech, USA), was additionally administered per each 100 kg (this is addition of 50 mg Zn/kg into feed mixture). The rabbits were divided into 4 experimental groups.

Every day, at the same time in the morning, the rabbits in the group 3EG and 4EG received a dose with *Thymus vulgaris* L. (0.1% plant extract) into drinking water. Natural products of plant origin (in this case the extract of *Thymus vulgaris* L.) are still a major part of traditional medicine. The extract of *Thymus vulgaris* L. contains especially flavonoids (54%) diterpene (27%) and phenolic acids (19%), which are known to be antimicrobials and antioxidants. The overall mortality (Table 2) during our experiment was in the group 1EG 1 rabbit and in the group 3EG 2 rabbits, and could be caused by intake of the basal diet without zinc additive (Bioplex-Zn) and higher feed intake of starch (18.3%).

Table 2. Growth performance of rabbits in response to dietary supplementation with zinc from organic source Bioplex-Zn (mean  $\pm$  SD)

Parameter (n=24)	1EG	2EG	3EG	4EG
Initial weight (g)	1161 $\pm$ 119	1114 $\pm$ 136	1071 $\pm$ 124	1114 $\pm$ 149
Final weight (g)	2779 $\pm$ 300	2830 $\pm$ 268	2796 $\pm$ 208	2859 $\pm$ 344
Feed conversion ratio in g/g	3.51	3.04	3.22	3.23
Mortality (n)	1	0	2	0
Carcass yield (%)	55.65 $\pm$ 0.71	55.88 $\pm$ 2.23	54.92 $\pm$ 0.46	57.17 $\pm$ 0.71

1EG - Basal diet without supplementary Zinc; 2EG - Basal diet with supplementary with Bioplex - Zn at; 3EG - Basal diet without supplementary Zinc and with thyme plant extract 0.1% applied into water; 4EG - Basal diet with supplementary Bioplex-Zn combined with thyme plant extract 0.1% applied into drinking water.

Dietary supplementation of zinc to rabbits was carried out to determine its effects on growth of live weight and consumption of feed per unit of live weight growth. In rabbits, the majority of the digestive events occur in the caecum, i.e. at a place of a wide variety of microbial population and digestion. Most studies deal with the anaerobic - strictly and facultative - bacteria; their presence, counts and activity depend on the age of rabbits. Digestive disorders evoked by harmful bacteria are a predominant cause of mortality in many rabbits. Young animals are susceptible to the development of gastrointestinal diseases caused by feed-borne pathogens, particularly colibacillosis and clostridiosis (Takáčová et al., 2012). Most strains of *Escherichia coli* are a harmless component of the intestinal flora, but some of them (especially enteropathogenic *E. coli*) are primary intestinal pathogens. Highest numbers of bacteria were recorded in the faeces and were



almost in numbers as in the start of experiment except for coliform bacteria and *Pseudomonads* that were higher at the end of the experiment than in start - zero faecal samples. However, coagulase-negative staphylococci (CoNS), coagulase-positive staphylococci (CoPS), Lactic acid bacteria, *Clostridium*-like remained unchanged (Table 4). The counts of enterococci in the start reached 3.79 log<sub>10</sub> CFU.g<sup>-1</sup> and at the end of the experiment they were higher in all groups but still within the normal incidence of *Enterococci* in the faeces of animals (4.68-5.62 log<sub>10</sub> CFU.g<sup>-1</sup>). The numbers of the Gram-negative bacterial and *Pseudomonads* counts were higher than in the first-background sample, this may be affected by the summer period when the higher temperatures and outdoors, and despite the air-conditioned hall, it could have an impact on microbiota (Lauková et al., 2018).

Table 3. The effect of dietary zinc supplementation on selected processing technology parameters and chemical characteristic of MLD muscles 24 h post mortem (mean ± SD).

Characteristic (n=6)	1EG	2EG	3EG	4EG
Water g.100g <sup>-1</sup>	74.13±0.21	73.39±0.44	74.13±0.41	73.90±0.43
Protein g.100g <sup>-1</sup>	23.25±0.32	23.27±0.28	23.23±0.22	23.43±0.36
Σ EAA g.100g <sup>-1</sup>	10.06±0.63	10.01±0.74	10.59±1.13	10.86±0.66 <sup>a,b</sup>
Fat g.100g <sup>-1</sup>	2.08±0.22	2.24±0.29	2.23±0.30	2.22±0.27
SFA % of Σ fatty acid content	35.01±0.62	35.10±1.30	34.78±0.85	35.07±1.11
MUFA % of Σ fatty acid content	49.79±0.19	49.33±1.01	49.77±1.12	49.84±1.46
PUFA % of Σ fatty acid content	11.68±0.59	11.65±1.42	11.83±0.97	12.02±0.91
Color L	51.21±2.72	48.96±3.77	50.25±2.99	49.25±2.79
Electric conductivity μS.cm <sup>-1</sup>	0.80±0.20	0.89±0.46	2.50±1.33 <sup>a,b</sup>	1.77±0.81
Cholesterol g.100g <sup>-1</sup>	0.31±0.05	0.33±0.05	0.36±0.03	0.33±0.05
pH <sub>24</sub>	5.68±0.07	5.75±0.04	5.70±0.08	5.69±0.07
WHC g.100g <sup>-1</sup>	25.33±3.85	24.61±2.59	29.05±1.80 <sup>a,b</sup>	26.91±2.85
EV (kJ.100g <sup>-1</sup> )	467.66±3.8	461.62±11.8 <sup>a</sup>	472.79±9.63	453.11±13.9

a,b = Means with different superscripts within column differ significantly (p < 0.05); Σ EAA = Total essential amino acids

SFA = saturated fatty acids, include C8:0, C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C20:0, C22:0, C24:0

MUFA = monounsaturated fatty acids, include C16:1 n-7, C18:1 n-9c, C18:1 n-9t, C22:1

PUFA = polyunsaturated fatty acids, include C18:2 n-6, C18:3 n-3, C20:4 n-6, C20:5 n-3, C22:5 n-6, C22:6 n-3

WHC = Water holding capacity

Table 4. Selected bacterial groups in faeces of rabbits (log<sub>10</sub> CFU.g<sup>-1</sup> ± SD)

Bacterial strains (n=24)	The counts on Day 0-1	The bacterial counts on day 42			
		1EG	2EG	3EG	4EG
<i>Enterococci</i>	3.792±0.75	5.28±0.56	5.55±0.11 <sup>ba***</sup>	5.62±0.12 <sup>ca***</sup>	4.68±0.93
<i>Lactic acid bacteria</i>	4.666±0.93	5.08±1.01	5.41±1.08	4.62±0.92	4.79±0.95
<i>CoPS</i>	4.360±0.87	5.29±0.57	5.29±1.05	5.16±0.32	5.42±0.83
<i>CoNS</i>	4.394±0.87	4.71±0.94	4.67±0.93	4.69±0.94	5.66±0.13 <sup>a**</sup>
<i>Coliform bacteria</i>	3.779±0.87	6.91±1.38 <sup>a***</sup>	7.42±1.48 <sup>a***</sup>	6.89±1.37 <sup>a***</sup>	5.46±1.08 <sup>a***</sup>
<i>Pseudomonads</i>	5.206±1.20	7.43±1.48 <sup>a***</sup>	7.94±1.58 <sup>a***</sup>	7.79±1.55 <sup>a***</sup>	7.05±1.41 <sup>a***</sup>
<i>Clostridia</i>	4.039±0.81	5.35±1.07	4.72±0.94	5.65±1.41	5.42±1.08

1EG - Basal diet without supplementary Zinc; 2EG - Basal diet with supplementary with Bioplex - Zn at; 3EG- Basal diet without supplementary Zinc and with thyme plant extract 0.1% applied into drinking water; 4EG - Basal diet with supplementary Bioplex-Zn combined with thyme plant extract 0.1% applied into drinking water; CoPS coagulase-positive staphylococci; CoNS coagulase-negative staphylococci; \*P<0.05; \*\*P<0.01,\*\*\*P<0.001

The number of enterococci in the caecum was lower than in the faeces and in all the groups (Table 5); both in the control (1EG) and in the experimental groups (2, 3, 4) and were lower in background-first samples as well. It appears that the antagonistic effect of thyme also appeared in caecum when we recorded a significant decrease in enterococci in the 3<sup>rd</sup> EG compared to the 4<sup>th</sup> EG (<sup>cd</sup>P<0.001) as well as in the 3<sup>rd</sup> EG versus the 2<sup>nd</sup> EG (<sup>cb</sup>P<0.001); however, in the 4<sup>th</sup> EG, in addition to basal diet and thyme and Zn, thyme did not show an antagonistic effect. In contrast, Zn groups were found to have a higher number of enterococci (<sup>ab</sup>, <sup>ad</sup>P<0.001). Also, CoNS numbers were significantly lower in 3EG compared to 4EG (<sup>ab</sup>P <0.01). In the thyme group only (3<sup>rd</sup> EG), the CoPS was lower than the 4<sup>th</sup> EG as well as the 2<sup>nd</sup> EG (<sup>ba</sup>P<0.01; <sup>bc</sup>P<0.001). Even with lactic acid-producing bacteria, we found a significant decrease in the 3<sup>rd</sup> EG of thyme versus 4<sup>th</sup>EG, where both Zn and thyme as opposed to 2<sup>nd</sup> EG (with zinc, <sup>abc</sup>P<0.001). Clostridial counts were higher, but remained uncoated, respectively unchanged. The number of coliforms decreased in all groups compared to the 1EG (<sup>ba</sup>P<0.05, <sup>ca</sup>P<0.001, <sup>da</sup>P<0.001), also dropped compared to the 3<sup>rd</sup> EG with 2. 4., 2. and 3. with 4. (<sup>cb</sup>P<0.01, <sup>db</sup>P<0.001, <sup>cd</sup>P<0.01). *Pseudomonas spp.* were significantly decreased in 3<sup>rd</sup> EG compared to 1EG (<sup>ca</sup>P<0.05), in 4<sup>th</sup>EG compared to 1EG (<sup>da</sup> P<0.01), and in the 3<sup>rd</sup> and 4<sup>th</sup> EG with the 2<sup>nd</sup> EG(<sup>cb</sup>, <sup>db</sup>P<0.01, 0.001).

Table 5. Selected bacterial groups in caecum of rabbits (log 10 CFU.g<sup>-1</sup> ± SD)

Bacterial strains (n=6)	The bacterial counts on day 42			
	1EG	2EG	3EG	4EG
<i>Enterococci</i>	3.23±0.53 <sup>c***</sup>	2.23±0.04 <sup>c*</sup>	1.59±0.03	2.43±0.40 <sup>a*</sup>
<i>Lactic acid bacteria</i>	2.96±0.49 <sup>c***</sup>	2.38±0.04 <sup>c*</sup>	1.38±0.02	2.35±0.04
<i>CoPS</i>	2.58±0.43 <sup>d*</sup>	3.25±0.06 <sup>d**</sup>	1.92±0.32	1.79±0.29
<i>CoNS</i>	2.79±0.46 <sup>c**</sup>	2.85±0.47 <sup>c**</sup>	1.43±0.24	2.76±0.46
<i>Coliform bacteria</i>	5.83±0.97 <sup>a*</sup>	5.08±0.84 <sup>d*</sup>	4.27±0.71	3.06±0.51
<i>Pseudomonads</i>	5.24±0.87 <sup>cd*</sup>	4.66±0.78	3.67±0.63	3.64±0.60
<i>Clostridiae</i>	4.45±0.77	4.37±0.87	4.63±0.77	4.50±0.75

\*P<0.05; \*\*P<0.01,\*\*\*P<0.001

The dressing percent was calculated as the ratio of dressed weight to the live weight. Because of its low contents of fat and cholesterol as well as the high proportion of polyunsaturated fatty acids, rabbit meat is generally considered a “healthy” meat. Results of selected meat quality parameters (content of water, content of proteins, fat, ash, cholesterol and parameter of electric conductivity, pH, colour and water holding capacity) are presented in Table 3. Application of Bioplex-Zn and the plant extract *Thymus vulgaris* to rabbits had no effect on total water, protein, fat and differences among the groups were insignificant (P>0.05). In our study low ash content was measured in comparison to statements by other authors (Chrastinová et al., 2010). Concentration of cholesterol was increased in experimental groups in comparison with the control group, but differences were not significant (P>0.05). The pH of meat of rabbits in experimental group 1EG was lower when compared to the 2EG, but the differences were not significant (P>0.05). The pH value depends on the balance of muscle energy metabolism and represents a key role in the maintenance of the meat quality during storage. It determines the environmental microbial balance, because of the bacteriostatic effects of low pH on meats (Dalle Zotte et al., 2013; Pogany Simonová et al., 2010). In our study, lower pH<sub>24</sub> values (in the range 5.68 - 5.75) were obtained,

which could be explained by depletion of glycogen reserve in muscles during refrigeration and by a longer storage time. Meat pH affects many meat properties, including the water holding capacity, muscle fat content, and colour. Losses of water in meat lead to increased pH and decrease of muscle protein, which is closer to the isoelectric point and this results in a lower hydration level. Electric conductivity parameter was increased in each experimental group (in 3EG the highest) against the 1EG and 2EG, differences were significant ( $P \leq 0.05$ ). Colour L parameter was slightly decreased in experimental groups in comparison to the 1EG (in 2EG the lowest). There is also a positive correlation between water holding capacity and intramuscular fat content (Hernández et al. 2000) as well as the ultimate pH (Lambertini et al. 1996).

The essential amino acid composition is one of the most important nutritional qualities of protein. Nowadays, histidine is considered to be an essential amino acid because of the effects on haemoglobin concentrations (Report of a Joint WHO/ FAO/ UNU Expert Consultation (2007)). Although tryptophan was not detected, according to all of the detected amino acid scores, the protein in MLD muscle was well-balanced in essential amino acid composition and is of high quality. No negative influence was observed on growth performance, selected chemical characteristic of meat in MLD muscle in rabbits' meat and carcass yield. A positive influence of both substances was noted on animal health; particularly favourable effects have been manifested in gastro-intestinal tract.

## CONCLUSIONS

The experimental results show that dietary supplementation of zinc to rabbits was carried out to determine its effects on growth of live weight and consumption of feed per unit of growth weight. No significant differences were observed in mean of the carcass yield % in the tested variants. The beneficial effect of combination of the *Thymus vulgaris* plant extract and zinc additive administration was manifested by the anti-coccidian effect in rabbits. Antibacterial effect of thyme was shown by significant reduction of coliforms in EGs ( $P < 0.05$ ;  $P < 0.01$ ) and *Eimeria oocysts* were reduced in EGs compared to control. The dietary supplementation of organic zinc source and of combination of the *Thymus vulgaris* plant extract in drinking water is effective in improving the energy value and amino acid composition of rabbit meat and had positive effect on health status of rabbits.

## ACKNOWLEDGEMENTS

The work was supported by the project of APVV-0667-12 Zinc in animal nutrition and consumer safety and Vega 2/0006/17. The authors would like also thank to Mr. Ján Pecho, Mr. Igor Matusica, Dr. Rastislav Jurčik and Dr. Ľubomír Ondruška for their excellent technical assistance and co-operation in experiments.

## REFERENCES

- AOAC (2005). Official methods of analysis, 18<sup>th</sup> Ed. Association of official analytical chemists, Gaithersburg, USA.
- CHRASTINOVÁ, L., CHRENKOVÁ, M., LAUKOVÁ, A., POLÁČIKOVÁ, M., SIMONOVÁ, M., SZABÓOVÁ, R., STROMPFOVÁ, V., ONDRUŠKA, Ľ., CHLEBEC, I., PARKÁNYI, V., RAFAY, J. and VASILKOVÁ, Z. (2010)



Influence of selected phytoadditives and probiotics on zootechnical performance, caecal parameters and meat quality of rabbits. *Archivos Zootecnia*, **13**: 30-35.

**CHRASTINOVÁ, L., ČOBANOVÁ, K., CHRENKOVÁ, M., POLÁČIKOVÁ, M., FORMELOVÁ, Z., LAUKOVÁ, A., ONDRUŠKA, L., POGÁNY SIMONOVÁ, M., STROMPFOVÁ, V., MLYNEKOVÁ, Z., KALAFOVÁ, A. and GREŠÁKOVÁ, L.** (2016) Effect of dietary zinc supplementation on nutrients digestibility and fermentation characteristics of caecal content in physiological experiment with young rabbits. *Slovak Journal of Animal Science*, **49**: 23-31.

**DALLE ZOTTE, A., SARTORI, A., BOHATIR, P., REMIGNON, H. and RICCI, R.** (2013) Effect of dietary supplementation of Spirulina (*Arthrospira platensis*) and Thyme (*Thymus vulgaris*) on growth performance, apparent digestibility and health status of companion dwarf rabbits. *Livestock Science*, **152**: 182-191.

**HAŠEK, O. and PALANSKÁ, O.** (1976) Determination of water holding capacity in meat by instruments at constant pressure. *Poultry Industry*, **18**: 228-233.

**HERNÁNDEZ, P., PLA, M., OLIVER, M.A. and BLASCO, A.** (2000) Relationships between meat quality measurements in rabbits fed with three diets of different fat type and content. *Meat Science*, **55**: 379-384.

**LAMBERTINI, L., BERGOGLIO, G., MASOERO, G. and GRAMENZI, A.** (1996) Comparison between Provisal and Hyla rabbit strains. I. Slaughtering performances and muscle composition. Proceedings of 6<sup>th</sup> WRSA Congress, Toulouse, pp. 195-199.

**LAUKOVÁ, A., CHRASTINOVÁ, L., ČOBANOVÁ, K., KANDRIČÁKOVÁ, A., BINO, E., FORMELOVÁ, Z., GANCARČIKOVÁ, S., ŠTRKOLCOVÁ, G., PARKÁNYI, V. and GREŠÁKOVÁ, L.** (2018) Microbiota and some parameters in broiler rabbits after zinc and thyme application. Book of abstracts of XXXIX Hygiene alimentorum Congress, Štrbské Pleso, pp. 70.

**MC CALL, K.A., HUANG, C. and FIERKE, C.A.** (2000) Function and mechanism of zinc metalloenzymes. *The Journal of Nutrition*, **130**: 1437-1446.

**MINISTRY OF AGRICULTURE, FISHERIES AND FOOD.** (1986) Manual of veterinary parasitological laboratory techniques, 3<sup>rd</sup> ed. London: Her Majesty's Stationery Office (HMSO), pp. 160.

**PLA, M. and DALLE ZOTTE, A.** (2000) Harmonisation of criteria and methods used in rabbit meat research. *World Rabbit Science*, **8**: 539-545.

**POGÁNY SIMONOVÁ, M., CHRASTINOVÁ, L., MOJTO, J., LAUKOVÁ, A., SZABÓOVÁ, R. and RAFAY, J.** (2010) Quality of rabbit meat and phyto-additives. *Czech Journal of Food Science*, **28**: 161-167.

**POWELL, S.R.** (2000) The antioxidant properties of zinc. *The Journal of Nutrition*, **130**: 1447S-1454S.

**RAFAY, J., MOJTO, J. and PALANSKÁ, O.** (1999) Characteristics of meat quality of domestic rabbit. *Agriculture*, **45**: 388-396.

**RUBIO, C., GONZÁLEZ, D., MARTÍN-IZQUIERDO, R.E., REVERT, C., RODRÍGUEZ, I. and HARDISSON, A.** (2007) El zinc: oligoelemento esencial. *Nutrición Hospitalaria*, **22**: 101-107.

**STEFANIDOU, M., MARAVELIAS, C., DONA, A. and SPILIOPOULOU, C.** (2006) Zinc: a multipurpose trace element. *Archives of Toxicology*, **80**: 1-9.

**STN 570185** (1962) Slovak Technical Standards. Examination of meat, meat products and canned meat and prepared meats in cans. Chemical and physical methods. Bratislava, Slovakia.

**SUTTLE, N.F.** (2010) Mineral nutrition of livestock. *CABI Publishing, Wallingford, Oxfordshire*, No. 4.

**TAKÁČOVÁ, J., RYZNER, M., PLACHÁ, I., ČOBANOVÁ, K., FAIXOVÁ, Z. and FAIX, Š.** (2012) *Thymus vulgaris* essential oil in poultry nutrition. Book of abstracts of 25<sup>th</sup> days of animal physiology, Košice, pp. 72.